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MOLECULAR APPROACHES FOR DETECTING CHROMOSOME ALTERATIONS AND FORWARD MUTATIONS IN MAMMALIAN CELLS.

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Genetic damage resulting from chemical and radiation exposure to mammalian cells is manifested in a rich array of events including single and double strand breakage, alkylation, cross-linking, thymidine dimerization, and base removal. These initial lesions typically undergo (mis)repair leading to a broad spectrum of microscopic and sub-microscopic mutational events including base pair substitutions, frame shifts, deletions, chromatid and chromosome exchanges, and aneuploidy. For large-scale events that require analysis of major portions of the genome, fluorescence *in situ* hybridization is a powerful method for quantifying structural and numerical chromosome changes in metaphase and interphase cells. A common application known as “chromosome painting” labels entire chromosomes. Painting probes exist for a number of species including humans, mice and rats, and are useful for detecting translocations which are stable exchanges that survive cell division and are commonly found in tumor cells. Chromosome-region probes have been used to decipher the contents of micronuclei, and have clarified our understanding of the spectrum of mechanisms that lead to abnormalities of cell division. Detection of sub-microscopic deletions, point mutations and insertions involves analysis of specific genes. A typical approach involves cell culture under conditions that favor the growth of mutants (e.g., hprt, aprt, and tk loci), followed by isolation and characterization of DNA from the gene of interest. Other assays reveal altered or missing surface markers. The erythrocyte-based glycophorin A and the intestinal cell dlb-1 assays are useful for *in vivo* exposure assessments in humans and mice, respectively. These methods do not require cell culture, and are advantageous for screening large numbers of cells in tissues that cannot otherwise be readily examined. However, the methods suffer from the inability to clone the mutant cells and to characterize the mutations. The approaches for detecting chromosome alterations and forward mutations are complementary, and when used together they have significant potential for improving our understanding of genotoxicity. Work performed under the auspices of the US DOE by LLNL, contract No. W-7405-ENG-48.